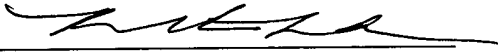


**REMARKS**

The foregoing amendments are made to insert the sequence identification numbers into the specification. No new matter has been added.

Respectfully submitted,

Date: 12/21/01

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**Version with markings to show changes made**

In the specification:

Paragraph beginning on page 8, line 13 has been amended as follows:

Figure 5 presents multiple sequence alignments of sequences with some similarities to *DWF5* (LBR-Human, SEQ ID NO:22; LBR-Rat, SEQ ID NO:23; LBR-Chicken, SEQ ID NO:24; S14R-Yeast, SEQ ID NO:25; S7R-Human, SEQ ID NO:26; S7R-Rat, SEQ ID NO:27; DWF5, SEQ ID NO:45). A consensus sequence is shown in the bottom row of the alignment. Dashes in the consensus mean <50% identity among the 7 sequences compared. Capital letters stand for residues conserved among all 7 sequences, whereas lower case letters mean 50% identity. Multiple sequence alignment was performed using PILEUP in the Genetics Computer Group software (Madison, WI) with a gap creation penalty of 4 and a gap extension parameter of 1.

Paragraph beginning on page 8, line 17 has been amended as follows:

Figure 7 presents the genomic sequence of the *DWF5* locus (polynucleotide SEQ ID NO:28, polypeptide SEQ ID NO:29) including annotations regarding the transcription start site (nucleotide 634), translation start site (nucleotide 671), exons (those portions of the sequences with corresponding polypeptide sequence) and introns (no corresponding polypeptide sequence indicated), sites of mutations corresponding to *dwf5-4* (polynucleotide SEQ ID NO:38, polypeptide SEQ ID NO:39), *dwf5-2* (polynucleotide SEQ ID NOS:32 and 34, polypeptide SEQ ID NOS:33 and 35), *dwf5-3* (polynucleotide SEQ ID NO:36, polypeptide SEQ ID NO:37), *dwf5-5* (polynucleotide SEQ ID NO:40, polypeptide SEQ ID NO:41), *dwf5-6* (polynucleotide SEQ ID NO:42, polypeptide SEQ ID NO:43), *dwf5-1* (polynucleotide SEQ ID NO:30, polypeptide SEQ ID NO:31), and translation products resulting from mis-spliced mRNAs.

Paragraph beginning on page 8, line 23 has been amended as follows:

Figure 8 shows a sequence of a DWF5 cDNA (SEQ ID NO:44) and the corresponding translation product (SEQ ID NO:45).

Paragraph beginning on page 50, line 28 has been amended as follows:

To determine the map position of a candidate S7R gene (GenBank accession number U49398), a CAPS marker (Konieczny and Ausubel, 1993) for the locus was created and the linkage of this polymorphism to *dwf5* was tested. Routine molecular techniques for DNA and RNA handling were performed according to Sambrook, et al. (1989). Some of the oligonucleotide sequences employed were as follows (sequences are given in the 5' to 3' direction):

DW5\_FF, GTGTGAGTAATTTAGGTCAACACAGATCA (SEQ ID NO:1);  
DW5\_LR, GGCTCGGTCTTTTGATGATTCCAACGTT (SEQ ID NO:2);  
DW5\_2F, TGTGGTAACCTAATAATTGACTTCTATT (SEQ ID NO:3);  
DW5\_2R, GGAGAAGTGTAGACAGAAGGCACCCACACT (SEQ ID NO:4);  
DW5\_3F, ATTGGAACACCATGGACATTGCACATGAC (SEQ ID NO:5);  
DW5\_4F, AGGGTCCAATATCTCCAGCCGGAACCGA (SEQ ID NO:6);  
DW5\_4R, GAAAATATTTACCCCAAGTGATCATAGA (SEQ ID NO:7);  
DW5\_5F, GGGTGCCTTCTGTCTACACTTCTCCAG (SEQ ID NO:8); and  
DW5\_5R, AAATGACGAGCCAATCCCCA (SEQ ID NO:9).

Paragraph beginning on page 54, line 24 has been amended as follows:

To confirm that the S7R gene encodes DWF5, the genomic DNA of Ws-2, En-2, and Estland wild types, were sequenced and compared to sequence obtained from the mutant alleles, described herein, of *dwf5*. Genomic DNA flanking the *dwf5* cDNA was isolated by thermal asymmetric interlaced PCR (Liu, et al., 1995). The following two sets of nested primers were used to amplify each of the 3' and 5' flanking DNAs (the oligonucleotide sequences are presented in the 5' to 3' orientation):

D5-3-1, TTACTCTGATTGCTGACAATATTCGGGTTTTG (SEQ ID NO:10);  
D5-3-2, GTAAAAAGGTATGGGAAATATTGGAAGCTGTAT (SEQ ID NO:11);  
D5-3-3, ATTGTAACGAAGTCTGTTGTTCTCATTTTCTAC (SEQ ID NO:12);  
D5-5-1, AGGAGCCAGAAAAGTGTGCGAGTC (SEQ ID NO:13);  
D5-5-2, CAGGAGAATGACGAAAGGTGGACA (SEQ ID NO:14); and  
D5-5-3, TGGACAGAAGGCGAGAAGCGATAA (SEQ ID NO:15).

Paragraph beginning on page 55, line 14 has been amended as follows:

RT-PCR was carried out to synthesize *DWF5* cDNA from the wild-type and *dwf5* mutants. Total RNA was subject to DNAase I treatment (Boehringer Mannheim-Roche, Indianapolis, IN) to remove genomic DNA. RNA was purified from the DNAase reaction using a RNEASY MINI KIT (Qiagen, Santa Clarita, CA). cDNA was first synthesized using reverse transcriptase (BRL, Gaithersburg, MD) with a poly T primer called A1T17 (5'-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTTTT-3') (SEQ ID NO:16). *dwf5* cDNA spanning the whole coding region was amplified using primers D5WKPN-F (5' ATCGGTACCAAGCAGAAGAAGAAAATGGCGGAG-3') (SEQ ID NO:17) and D5BAM-5 (5'-ATCGGATCCGCATTTTTGTTTTGGCTCGGTCTTTTGA-3') (SEQ ID NO:18).

Paragraph beginning on page 57, line 3 has been amended as follows:

In Figure 5, about 200 N-terminal amino acids of all LBRs and 29 C-terminal amino acids of the chicken LBR were truncated to maximize alignment. Amino acid residues conserved more than 50% among the 7 compared sequences are shown in inverse characters. Amino acid residues conserved among sterol  $\Delta^7$  reductases (S7R) are boxed. Positions for the Arabidopsis *dwf5* mutations are annotated with filled triangles and described underneath. Mutations causing Smith-Lemli-Opitz syndrome (SLOS) are shown with filled circles according to Fitzky et al. (1998). "fs" refers to frameshift

mutations. Introns are indicated with open circles (Arabidopsis) or open triangles (human DHCR7). Previously reported domains, EFGGxxG (SEQ ID NO:20) and LLxSGWWGxxRH (SEQ ID NO:19), a newly identified S7R signature, and the mixed charge cluster are shaded and labeled. Dashes in protein sequences indicate gaps introduced to maximize alignment. A consensus sequence is shown in the bottom row of the alignment. Dashes in the consensus mean <50% identity among the 7 sequences compared. Capital letters stand for residues conserved among all 7 sequences, whereas lower case letters mean 50% identity. Multiple sequence alignment was performed using PILEUP in the Genetics Computer Group software (Madison, WI) with a gap creation penalty of 4 and a gap extension parameter of 1.

Paragraph beginning on page 57, line 20 has been amended as follows:

As indicated in Figure 5, the previously reported LLxSGWWGxxRH (SEQ ID NO:19) and EFgGxxG (SEQ ID NO:20) signatures (Lecain, et al., 1996) (upper case for fully identical residues, lower case if conserved >50%, and x for variable residues) were identified at the N-terminus. In addition, it was found that the GrCLiWGrk (SEQ ID NO:21) signature is only conserved in  $\Delta^7$  reductases but missing in other reductases (Figure 5).

Paragraph beginning on page 58, line 5 has been amended as follows:

To summarize, the *dwf5* sequence was most similar to human and rat S7Rs. Yeast sterol  $\Delta^{14}$  and  $\Delta^{24}$  reductases, and the C-terminal 400 amino acids of human and chicken LBR also showed significant similarity (Figure 5). LBRs show identity with *dwf5* because the 400 amino acids of the C-terminal domain reportedly possess a  $\Delta^{14}$  reductase activity (Silve, et al., 1998), whereas the remaining N-terminal domain has been proposed to be involved in nuclear assembly during the cell cycle (Gant and Wilson, 1997). The combined function of the whole protein is as yet unclear. Lecain et al. (1996) first

identified two types of signature sequences in  $\Delta^7$  reductases. One group of amino acid residues, LLxSGWWGxxRH (SEQ ID NO:19), was commonly conserved in all of the sterol reductases, whereas the other group, EFGGxxG (SEQ ID NO:20), distinguished  $\Delta^7$  reductases from other sterol reductases (Figure 5). In addition to these, our sequence alignment revealed two additional domains both located in the C-terminal half of the protein. First, a signature consisting of GrCLiWGrk (SEQ ID NO:21) was only found in S7R sequences (Figure 5), suggesting a specific role in these enzymes. Second, an unusual cluster of charged amino acid residues was identified at the C-terminal end of all the reductase sequences. A mixed charge cluster (MCC) is highly conserved within the cut family of homeodomains proteins, suggesting an important role in this group of proteins (Brendel, et al., 1992). Similarly, it is likely that the terminal MCC domain of the sterol reductases plays a pivotal role for the proper function of the enzymes. In support of this, most of the *dwf5* mutations described in the examples below were directly associated with this domain. *dwf5-3* and *dwf5-5* carried premature stop codons in the MCC domain, resulting in strong mutations. *dwf5-4* contained a mutation changing a conserved Asp (D) to Asn (N) located at the starting region of the 6th transmembrane domain (Figure 5; Fitzky, et al., 1998). In relation to this, many of the mutations in human SLOS patients were found in or near the putative transmembrane domains (Fitzky, et al., 1998).